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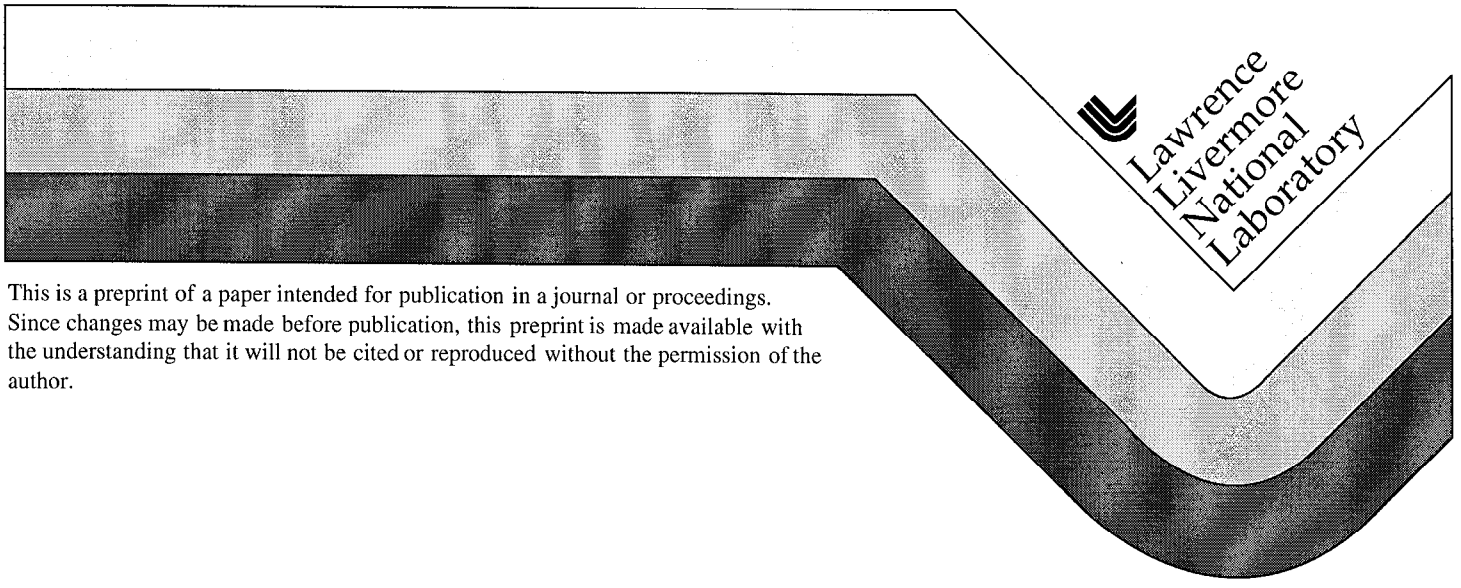

Polarization Sensitive Optical Coherence Tomography Detection Method

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ABSTRACT

This study demonstrates the potential of polarization sensitive optical coherence tomography (PS-OCT) for non-invasive in vivo detection and characterization of early, incipient caries lesions. PS-OCT generates cross-sectional images of biological tissue while measuring the effect of the tissue on the polarization state of incident light. Clear discrimination between regions of normal and demineralized enamel is first shown in PS-OCT images of bovine enamel blocks containing well-characterized artificial lesions. High-resolution, cross-sectional images of extracted human teeth are then generated that clearly discriminate between the normal and carious regions on both the smooth and occlusal surfaces. Regions of the teeth that appeared to be demineralized in the PS-OCT images were verified using histological thin sections examined under polarized light microscopy. The PS-OCT system discriminates between normal and carious regions by measuring the polarization state of the back-scattered 1310 nm light, which is affected by the state of demineralization of the enamel. Demineralization of enamel increases the scattering coefficient, thus depolarizing the incident light. This study shows that PS-OCT has great potential for the detection, characterization, and monitoring of incipient caries lesions.

1. INTRODUCTION

Although the prevalence of dental caries has declined over the past few decades, dental decay is still the leading cause of tooth loss in the USA[1-3]. The most common and difficult to detect early enamel lesions are occlusal (biting surfaces) pit and fissure, and approximal (contact surfaces between teeth) lesions in posterior teeth. If such lesions are detected early enough, it is likely that they can be arrested/reversed by topical fluoride or by low intensity laser irradiation without the need for surgical intervention[4]. Here we demonstrate a new technique for detection and imaging of caries lesions based on detection of depolarized light using polarization sensitive optical coherence tomography (PS-OCT). This technique discriminates between normal and carious regions of enamel by measuring the degree of polarization of back-scattered incident light, which is affected by the state of demineralization of the enamel. In addition to offering the potential for early caries detection, PS-OCT uses non-ionizing radiation, thus avoiding the cancer risks associated with radiographic detection of cavities.

Optical Coherence Tomography (OCT) is a non-invasive imaging technique that utilizes a short-coherence-length broadband light source to generate high-resolution (<20-μm) cross-sectional images of biological tissue [5]. Polarization sensitive OCT systems also measure the polarization state of the backscattered light, providing additional information about the optical properties of the tissue. To date, PS-OCT systems have been applied to elimination of birefringence related artifacts in tissue imaging and measurement of birefringence in various tissues including tendon, myocardium and teeth[6-8].

Baumgartner *et al* showed the first polarization resolved OCT images of teeth and investigated the potential of PS-OCT as a diagnostic of caries based on changes in birefringence[8]. However, we show that the analysis of the degree of depolarization of the incident light has greater potential than birefringence measurements for discriminating carious from normal tissue. We demonstrate discrimination of pre-carious lesions in tissue models and extracted human teeth as a function of depth using PS-OCT by measuring the depolarization of incident light caused by demineralized enamel. These results are then confirmed with histological imaging.

2. EXPERIMENTAL SETUP

The PS-OCT system, described in detail elsewhere[9] is based on a polarization sensitive Michelson white light interferometer as shown in Fig. 1. Circularly polarized low coherence light is focused on biological tissue in the sample arm. High resolution cross-sectional imaging and characterization of the tissue are then obtained by measuring the backscattered light intensity and polarization as a function of axial depth and transverse location in the tissue. A scanning retro-reflector varies the path length of the reference arm for each transverse location on the sample. An interferometric signal is detected when the distance to the reference and sample arm reflections is matched to within the source coherence length. The return light is split into orthogonal polarizations (horizontal and vertical) before being detected.

As shown in Fig. 1, a collimated beam from a 15 mW superluminescent diode source operating at a center wavelength of $\lambda_0 = 1310$ nm with a spectral bandwidth FWHM of $\Delta\lambda = 47$ nm is passed through a polarizer PBS1, providing 7.5 mW of horizontally polarized light. This light is split into the reference and sample arms of the Michelson interferometer by a non-polarizing beamsplitter. A quarter wave plate QW1 set at 22.5° to horizontal in the reference arm, rotates the polarization of the light by 45° upon reflection. A quarter wave plate QW2 set at 45° to horizontal in the sample arm, causes circularly polarized light to be incident upon the sample. After being reflected from the reference mirror and the sample the beams are recombined by the beamsplitter. A polarizing cube splits the recombined beam into its horizontal and vertical polarization components which are then coupled by single mode fiber optics onto two detectors D_H and D_V respectively. The light from the reference arm is polarized at 45° and therefore split evenly between the two detectors. The light returning from the sample arm has its polarization state altered by the tissue and is heterodyned with the reference light at the two detectors and demodulated by lock-in amplifiers. The resulting signals are squared to determine the power $P_H(z)$ and $P_V(z)$ in the two orthogonal polarization states scattered from the tissue as a function of depth z. The horizontally and vertically polarized light at the polarizing beamsplitter, $P_H(z)$ and $P_V(z)$, correspond to left and right circularly polarized light leaving the tissue. The total backscattered light versus penetration depth in the tissue is given by:

$$P_T(z) = P_V(z) + P_H(z) \tag{1}$$

and the degree of polarization of this light by:

$$\rho(z) = \frac{P_V(z) - P_H(z)}{P_V(z) + P_H(z)} \tag{2}$$

When there is no birefringence or depolarization in the tissue, $P_H(z) = 0$ and the degree of polarization $\rho(z)$ is equal to 1. Depolarization of the light spreads the light equally between the detectors, causing $\rho(z)$ to approach zero. Birefringence in the tissue can also affect this measurement of degree of polarization, causing the light to swing back and forth between the two detectors and leading to an oscillation in the value of $\rho(z)$ between 1 and –1 with tissue depth z. Cross-sectional images showing the intensity and polarization state of light backscattered from the enamel versus transverse location and penetration depth are generated by moving the tissue sample transversely with a motorized stage while scanning axially. Each image consists of 240 axial scans 4.5 mm in length separated transversely by 50 μm. The axial scans are acquired at a rate of 5 per second, generating a 4.5 mm by 12 mm image in 48 seconds. These images were then cropped appropriately to display the region of interest. To minimize noise, the signals $P_H(z)$ and $P_V(z)$ were averaged over 50 μm axial before calculating $\rho(z)$.

Both bovine enamel blocks and extracted human teeth were imaged with the system. The bovine blocks, 5 mm on a side, were cut from bovine incisors. These blocks were first serially polished with 6, 3, and 1-μm diamond suspensions to produce a smooth enamel surface appropriate for the formation of uniform and consistent caries-like lesions. Lesions were then created using a well characterized demineralizing solution[10] which forms lesions typical of the very early stages found in natural caries. After protecting half of the surface of each block with a thin coat of acid resistant varnish, the blocks were immersed individually in 40 mL of the buffer solution for 48 hours at 37°C. The buffer contained 0.1 mol/L lactate, 0.2% Carbopol (as a thickener and surface protector), hydroxyapatite at a 50% degree of saturation, with pH adjusted to 5.0. A zone of demineralization approximately 50 μm thick was thus produced on the polished surface of the unprotected half of the bovine enamel block. The acid resistant varnish was then removed, exposing the normal enamel on the other half.

Extracted human teeth were marked with a small (approximately 300 μm diameter) black paint dot near the top and the bottom of the tooth before obtaining the OCT scan. Each OCT scan was then taken through the two dots, which were used to ensure accurate alignment of the histological slice relative to the OCT image. After scanning, the teeth were sliced into 100 μm thick sections using a hard tissue microtome for observation under polarized light. Thin sections were imbibed with water and examined in a polarizing microscope (Olympus BH2) connected to an image analyzer with Bioquant software and a calibrated measuring system. Sections were examined in the brightfield mode with crossed polarizers and a red I plate with 500-nm retardation.

3. RESULTS

The ability of PS-OCT to detect caries lesions was initially demonstrated in bovine enamel blocks containing lesions generated artificially as described above. Cross-sectional PS-OCT backscattering intensity and polarization state images of these enamel blocks were taken such that half of each image was of normal enamel and half of demineralized enamel. Backscattering and degree of polarization images for a typical bovine enamel block are shown in Figure 2. Although the back-scattering intensity was only slightly affected by the demineralization, the polarization image clearly delineates the normal enamel from the demineralized region. This clear delineation was caused by the demineralized enamel depolarizing the incident light. It is also important to note that the polarization state measurement,

unlike the backscattered intensity measurement, is ratiometric and thus insensitive to fluctuations in signal intensity.

Having demonstrated the efficacy of PS-OCT for detection of demineralization in bovine enamel blocks with artificial demineralization, we next applied it to in vitro detection of naturally occurring caries in extracted human teeth. PS-OCT was used for caries detection in extracted human teeth and the results were compared with histological thin sections examined under polarized light. The cross-sectional PS-OCT image and corresponding histology shown in Fig. 3 were taken of an extracted tooth with an interproximal white spot lesion on the smooth surface. The scan extends from the top of the tooth at the top of the image to just above the cemento-enamel junction. The white spot lesion, visible in the thin section histology as a darkened region near the enamel surface, is clearly detected in the polarization image as indicated by the depolarization of the light within the box marking the lesion. Once again, the caries affects the intensity image, leading to a loss of signal or shadowing behind it in the enamel, but is more clearly seen in the polarization image. The healthy enamel above the box is highly birefringent as indicated by the black and white stripes (banding) caused by $\rho(z)$ varying from 1 to -1 in the polarization resolved image. Below the box there is little birefringence as indicated by the maintenance of polarization state $\rho(z)$ with depth. Enamel is birefringent because the index of refraction for light polarized along the axis of the enamel prisms is different from the index of refraction for light polarized perpendicular to the axis. The amount of birefringence in healthy enamel thus depends on the relative orientation between the enamel prisms and the incident probe light. The enamel prisms are made up of hundreds of individual crystals, are 4-6 μm in diameter , up to 3 mm long and extend from the dentin-enamel junction to the outer enamel surface like the spokes of a wheel. Below the white spot lesion in Fig. 3, where the axis of the prisms is parallel to the direction of propagation of the probe light, the two axes of polarization of the incident light are both perpendicular to the axis of the prisms and therefore minimal birefringence occurs. Near the top of the tooth, the prisms become more perpendicular to the probe light, leading to significant birefringence. This large variation in birefringence makes it difficult to use birefringence as an indicator of caries. Furthermore, we have not been able to detect birefringence changes associated with caries with our PS-OCT system.

After demonstrating the efficacy of PS-OCT for caries detection on the smooth surface of human teeth, we explored PS-OCT caries detection on the occlusal (biting) surface. Decay that is difficult to detect with standard methods often occurs on this surface in the pits and fissures. In fact, 80% of all new carious lesions today occur in these high risk areas[11]. PS-OCT caries detection on this surface was complicated somewhat by variations in birefringence across the tooth, but still quite successful. An extracted tooth containing caries was scanned with the PS-OCT system and then sliced in half and imaged to corroborate the PS-OCT results. Thin slice histology was not used in this case because the caries greatly reduced the structural integrity of the slices, making it difficult to obtain suitable thin sections. A photograph of the tooth cross-section along with the OCT images is shown in in Fig. 4. Once again, excellent correlation is seen between the carious regions in the photograph and OCT images. Depolarization is observed as a speckled gray area in the polarization resolved image, Fig. 4(c), throughout the boxed area containing the caries and is greatest in the center where the caries is most significant. Banding (black and white stripes) associated with birefringence is seen near the top and bottom of the tooth in this image. This banding is not desirable as it could lead to false positives if the birefringence changes the polarization state of the incident light just enough to look like depolarization. However, the birefringence effects can be eliminated through the use of multiple scans with different incident polarization states as shown in the Figure 4(d).

It is also important that a caries diagnosis system be able to distinguish degrees of demineralization so that the clinician can monitor lesions over time to determine if they are progressing or have been arrested. This capability is demonstrated on the occlusal surface shown in Figure 5. Visual inspection of this tooth indicated potential caries in the occlusal fissures at the center of the tooth with healthy enamel peripheral to those fissures. A set of three PS-OCT scans using the improved system (multiple scans) shows steadily increasing depolarization associated with demineralization as one moves toward the center of the tooth. This increasing depolarization is visible as a reduction in the depth to which the light remains polarized, leading to a narrowing of the black band at the surface of the tooth in the PS-OCT polarization image. Birefringence effects were eliminated in these images through the use of multiple (8) PS-OCT scans per image. It should be possible, as this technique is refined, to enhance the images to make them readily useable for clinical applications.

4. CONCLUSIONS

We have successfully demonstrated non-invasive detection and imaging of carious lesions with non-ionizing radiation in tissue models and in extracted human teeth. This was accomplished by using polarization sensitive optical coherence tomography (PS-OCT) to measure the depolarization of probe light versus penetration depth in the enamel. The depolarization was caused by increases in optical scattering associated with demineralization of the enamel. Histological sectioning confirmed the capability of the PS-OCT system for detection of caries and pre-caries lesions on both the smooth and occlusal surfaces. In addition, the results clearly demonstrate the potential of PS-OCT for assessing the severity of the caries.

5. ACKNOWLEDGMENTS

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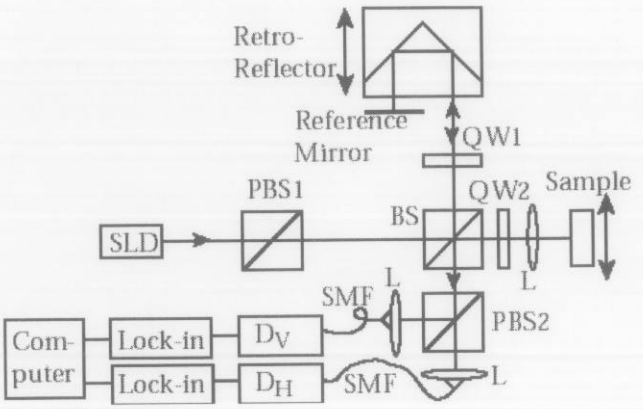

Figure 1. Schematic of polarization-sensitive OCT system: SLD - superluminescent diode; PBS - polarizing beamsplitter cube; BS - beamsplitter; QW - zero order quarter-wave plate; L - lens; SMF - single mode fiber; D_H and D_V - photodetectors collecting horizontally and vertically polarized light respectively.

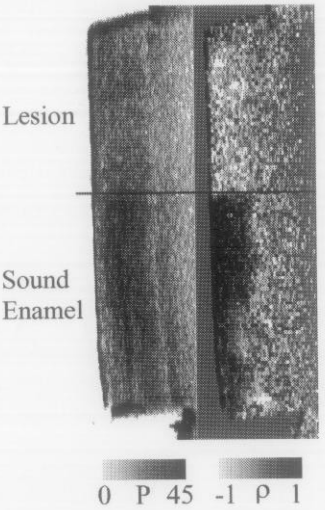

Figure 2. PS-OCT image of a bovine enamel block, which is 5 mm across with an artificially generated caries-like lesion above the horizontal black line (Left image– Intensity (dB), Right Image– Degree of Polarization). While the carious region is only weakly visible in the intensity image as a decrease in signal below the enamel surface, the carious region is clearly seen in the polarization image due to the depolarization of the incident light.

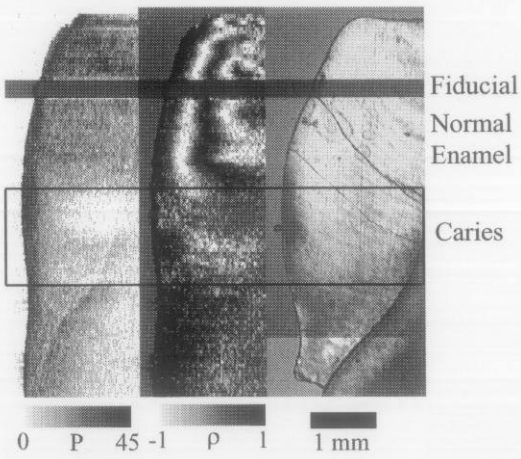

Figure 3. Cross-section of an extracted human tooth (from left to right, PS-OCT intensity, PS-OCT polarization, and histology) demonstrating PS-OCT detection of a white-spot lesion and histological confirmation highlighted by the black box. Although not easily visible in the PS-OCT intensity image, the white spot lesion causes rapid depolarization of the incident light visible as a transition from black to gray with depth in the PS-OCT polarization image. The location of an alignment paint spot is marked by the gray fiducial line.

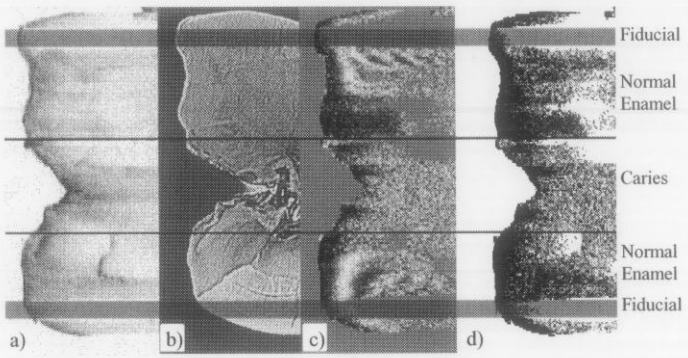

Figure 4. Cross-section of an extracted human tooth demonstrating PS-OCT detection of carious lesions on the occlusal (biting) surface and elimination of banding associated with birefringence: a) PS-OCT intensity image, b) Photograph of tooth cross-section, c) PS-OCT polarization image, d) PS-OCT polarization image with birefringence eliminated. The gray bands near the top and bottom of the images mark the locations of the alignment paint spots (fiducials).

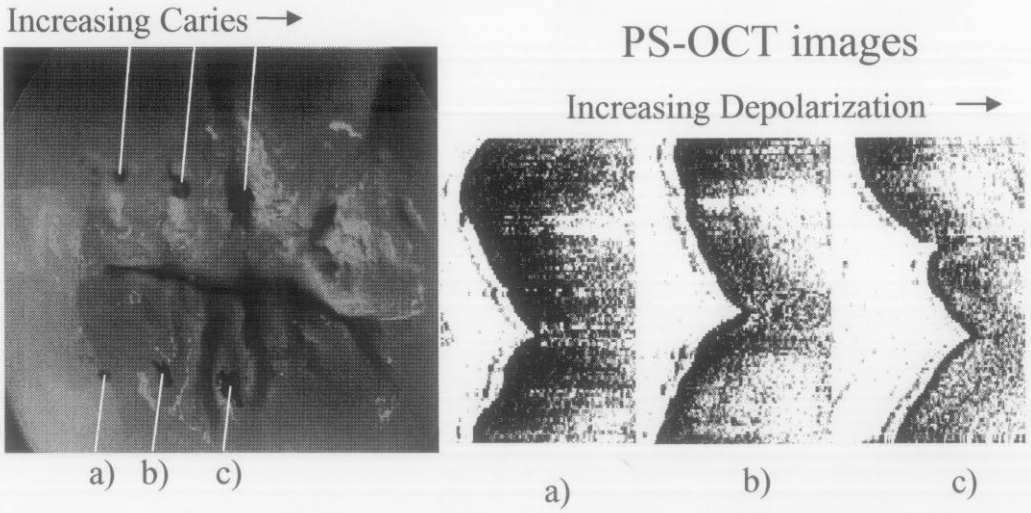

Figure 5. Demonstration of capability of PS-OCT for diagnosing severity of caries (Left image – photograph of occlusal surface of tooth imaged, Right images – PS-OCT polarization scans with birefringence eliminated.) Lines a), b) and c) mark the locations where the PS-OCT scans were taken. Visual inspection of the tooth indicated healthy enamel on the left side of the tooth where scan (A) was taken and potential caries at and to the right of scan (C). The depolarization in the PS-OCT images increases steadily from scan (A) to (C), consistent with the visual indications.